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Microbac Protocol

Evaluation of Antiviral Activity of Surfaces Coated by Antimicrobial Paint –

Feline calicivirus

Testing Facility
Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
Behr Paint Company
1801 E Saint Andrew Place
Santa Ana, CA 92705

November 10, 2020

Microbac Protocol: BEH.1a.11.04.20

Microbac Project: 1083-101

OBJECTIVE:

This test is designed to determine the antiviral activity of the Sponsor's coated surface materials. The protocol is applicable to surface paint/coating products that are intended for indoor use only. Feline calicivirus, a small, non-enveloped virus and a surrogate for human norovirus, will be tested in the protocol, as a representative hardest-to-kill virus.

The test is based on the EPA-approved Corning protocol MRID #51141402 "Protocol for Measuring Virucidal Efficacy of AM Paints", EPA's "Interim Method for Evaluating the Efficacy of Antimicrobial Surface Coatings" (10/02/2020), EPA's "Interim Method for the Evaluation of Bactericidal Activity of Hard, Non-porous Copper-Containing Surface Products" (01/23/20) with modifications to viral testing, and ASTM method E1053-20, "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces". This study will be performed in compliance with the EPA's Good Laboratory Practices (GLP) regulations, 40 CFR 160.

SUMMARY OF TESTING CONDITIONS:

Surface coupons (1" x 1" per piece) coated with either an antimicrobial paint ("treated carrier") or a control paint that contains no active ("coated control carrier") will be provided by the Sponsor. These coupons may have been "exposed" (i.e., subjected to a simulated wear procedure with different types of chemicals) or "unexposed" (i.e., no wear).

Each coupon will be UV irradiated to reduce bioburden and then inoculated with a viral inoculum (20 µL per carrier), which is then spread across the entire surface of the coupon. Upon inoculation, the coupon will be held at 20±1C and ambient relative humidity (RH) for 120±5 min in a Petri dish with the lid on. Then a 1.0-mL recovery medium (i.e., neutralizer) will be added onto the coupon to elute virus. The recovery sample will be serially diluted and assayed for the amount of infectious virus by a cell culture-based viral infectivity assay ($TCID_{50}$ assay). The amount of viable virus will be compared to that from a coated control carrier to determine the viral reduction by the treated carrier. A 3-log reduction window relative to the coated control carrier viral load shall be achieved. The coated control carrier load must be at least 4.8 log/carrier or 3-log above cytotoxicity.

Two lots of coated surface materials will be tested. Each lot will be tested with 5 types of base paints, with or without the antimicrobial active ingredients and with or without chemical "exposure". Two replicate carriers will be tested for each condition (see Table 1).

Table 1
Summary of Test Samples (1 of 4)

#	Lot #	Paint type	Copper additive?	Scrub exposure?	# carriers	Designation	abbreviation
1 A/B	1	2190	NO	NO	2	Unexposed control carrier – 2190	UC - 2190
2 A/B		2190-O			2	Unexposed control carrier – 2190-O	UC – 2190-O
3 A/B		2190-I			2	Unexposed control carrier – 2190-I	UC – 2190-I
4 A/B		2190-B			2	Unexposed control carrier – 2190-B	UC – 2190-B
5 A/B		3193			2	Unexposed control carrier – 3193	UC - 3193
6 A/B		2190		Cleaner	2	Cleaner-exposed control carrier – 2190	Cleaner EC - 2190
7 A/B		2190-O			2	Cleaner-exposed control carrier – 2190-O	Cleaner EC – 2190-O
8 A/B		2190-I			2	Cleaner-exposed control carrier – 2190-I	Cleaner EC – 2190-I
9 A/B		2190-B			2	Cleaner-exposed control carrier – 2190-B	Cleaner EC – 2190-B
10 A/B		3193			2	Cleaner-exposed control carrier – 3193	Cleaner EC - 3193
11 A/B		2190		Quat	2	Quat-exposed control carrier – 2190	Quat EC - 2190
12 A/B		2190-O			2	Quat-exposed control carrier – 2190-O	Quat EC – 2190-O
13 A/B		2190-I			2	Quat-exposed control carrier – 2190-I	Quat EC – 2190-I
14 A/B		2190-B			2	Quat-exposed control carrier – 2190-B	Quat EC – 2190-B
15 A/B		3193			2	Quat-exposed control carrier – 3193	Quat EC - 3193

Table 1
Summary of Test Samples (2 of 4)

#	Lot #	Paint type	Copper additive?	Scrub exposure?	# carriers	Designation	abbreviation
16 A/B	1	2190	YES	NO	2	Unexposed treated carrier – 2190	UT - 2190
17 A/B		2190-O			2	Unexposed treated carrier – 2190-O	UT – 2190-O
18 A/B		2190-I			2	Unexposed treated carrier – 2190-I	UT – 2190-I
19 A/B		2190-B			2	Unexposed treated carrier – 2190-B	UT – 2190-B
20 A/B		3193			2	Unexposed treated carrier – 3193	UT - 3193
21 A/B		2190		Cleaner	2	Cleaner-exposed treated carrier – 2190	Cleaner ET - 2190
22 A/B		2190-O			2	Cleaner-exposed treated carrier – 2190-O	Cleaner ET – 2190-O
23 A/B		2190-I			2	Cleaner-exposed treated carrier – 2190-I	Cleaner ET – 2190-I
24 A/B		2190-B			2	Cleaner-exposed treated carrier – 2190-B	Cleaner ET – 2190-B
25 A/B		3193			2	Cleaner-exposed treated carrier – 3193	Cleaner ET - 3193
26 A/B		2190		Quat	2	Quat-exposed treated carrier – 2190	Quat ET - 2190
27 A/B		2190-O			2	Quat-exposed treated carrier – 2190-O	Quat ET – 2190-O
28 A/B		2190-I			2	Quat-exposed treated carrier – 2190-I	Quat ET – 2190-I
29 A/B		2190-B			2	Quat-exposed treated carrier – 2190-B	Quat ET – 2190-B
30 A/B		3193			2	Quat-exposed treated carrier – 3193	Quat ET - 3193

Table 1
Summary of Test Samples (3 of 4)

#	Lot #	Paint type	Copper additive?	Scrub exposure?	# carriers	Designation	abbreviation
31 A/B	2	2190	NO	NO	2	Unexposed control carrier – 2190	UC - 2190
32 A/B		2190-O			2	Unexposed control carrier – 2190-O	UC – 2190-O
33 A/B		2190-I			2	Unexposed control carrier – 2190-I	UC – 2190-I
34 A/B		2190-B			2	Unexposed control carrier – 2190-B	UC – 2190-B
35 A/B		3193			2	Unexposed control carrier – 3193	UC - 3193
36 A/B		2190	YES	NO	2	Unexposed treated carrier – 2190	UT - 2190
37 A/B		2190-O			2	Unexposed treated carrier – 2190-O	UT – 2190-O
38 A/B		2190-I			2	Unexposed treated carrier – 2190-I	UT – 2190-I
39 A/B		2190-B			2	Unexposed treated carrier – 2190-B	UT – 2190-B
40 A/B		3193			2	Unexposed treated carrier – 3193	UT - 3193

Table 1
Summary of Test Samples (4 of 4) – Cytotoxicity, Neutralization and Other Controls

#	Lot #	Paint type	Copper additive?	Scrub exposure?	# carrier	Cytotoxicity Control	Neutralization Control
41	1	2190	YES	NO	1	CT - UT - 2190	NE - UT - 2190
42		2190-O			1	CT - UT – 2190-O	NE - UT – 2190-O
43		2190-I			1	CT - UT – 2190-I	NE - UT – 2190-I
44		2190-B			1	CT - UT – 2190-B	NE - UT – 2190-B
45		3193			1	CT - UT - 3193	NE - UT - 3193
46		2190		Cleaner	1	CT - Cleaner ET - 2190	NE - Cleaner ET - 2190
47		2190-O			1	CT - Cleaner ET – 2190-O	NE - Cleaner ET – 2190-O
48		2190-I			1	CT - Cleaner ET – 2190-I	NE - Cleaner ET – 2190-I
49		2190-B			1	CT - Cleaner ET – 2190-B	NE - Cleaner ET – 2190-B
50		3193			1	CT - Cleaner ET - 3193	NE - Cleaner ET - 3193
51		2190		Quat	1	CT - Quat ET - 2190	NE - Quat ET - 2190
52		2190-O			1	CT - Quat ET – 2190-O	NE - Quat ET – 2190-O
53		2190-I			1	CT - Quat ET – 2190-I	NE - Quat ET – 2190-I
54		2190-B			1	CT - Quat ET – 2190-B	NE - Quat ET – 2190-B
55		3193			1	CT - Quat ET - 3193	NE - Quat ET - 3193
56	2	2190		NO	1	CT - UT - 2190	NE - UT - 2190
57		2190-O			1	CT - UT – 2190-O	NE - UT – 2190-O
58		2190-I			1	CT - UT – 2190-I	NE - UT – 2190-I
59		2190-B			1	CT - UT – 2190-B	NE - UT – 2190-B
60		3193			1	CT - UT - 3193	NE - UT - 3193
61	N/A				1	Glass Plate Recovery Control	
63	N/A				1	Viral Stock Titer Control	
64	N/A				1	Cell Viability Control	

MATERIALS:

A. Test, control and reference substances will be supplied by the Sponsor of the study. Microbac will append the Sponsor-provided Certificate(s) of Analysis (CoA) to this study report, as per CFR 40.160.105:

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined and shall be documented by the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis.

The test substance will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test substance such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures Microbac testing facility management that the test substance has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

Microbac will retain all unused test substances for a period of one year upon completion of the test, and then discard them in a manner that meets the approval of the safety officer or return them to the Sponsor. The test materials and the paper records will be retained in accordance to FIFRA. Microbac will contact the Study Sponsor to arrange for transfer of records when/if the test substance is returned to the Sponsor.

B. Materials supplied by Microbac, including, but not limited to:

1. Challenge virus (requested by the sponsor of the study):
   - Feline calicivirus (surrogate for Human Norovirus), Strain: F9, ATCC VR-782

2. Host cell line:
   - CrFK, ATCC CCL-94

3. *(If required)* Sephadex or Sephacryl columns

4. Other laboratory equipment and supplies

5. Media and reagents:
   Media and reagents relevant to the virus-host system and test substance being tested will be documented in the first project sheet and data pack.

B. Materials to be supplied by the Sponsor:

Lot #1

• Lot #1	2190	No copper	No "exposure"	4 carriers
• Lot #1	2190-O	No copper	No "exposure"	4 carriers
• Lot #1	2190-I	No copper	No "exposure"	4 carriers
• Lot #1	2190-B	No copper	No "exposure"	4 carriers
• Lot #1	3193	No copper	No "exposure"	4 carriers
• Lot #1	2190	No copper	Exposed with cleaner	4 carriers
• Lot #1	2190-O	No copper	Exposed with cleaner	4 carriers
• Lot #1	2190-I	No copper	Exposed with cleaner	4 carriers
• Lot #1	2190-B	No copper	Exposed with cleaner	4 carriers
• Lot #1	3193	No copper	Exposed with cleaner	4 carriers
• Lot #1	2190	No copper	Exposed with quat	4 carriers
• Lot #1	2190-O	No copper	Exposed with quat	4 carriers
• Lot #1	2190-I	No copper	Exposed with quat	4 carriers
• Lot #1	2190-B	No copper	Exposed with quat	4 carriers
• Lot #1	3193	No copper	Exposed with quat	4 carriers

• Lot #1	2190	w/ copper	No "exposure"	6 carriers
• Lot #1	2190-O	w/ copper	No "exposure"	6 carriers
• Lot #1	2190-I	w/ copper	No "exposure"	6 carriers
• Lot #1	2190-B	w/ copper	No "exposure"	6 carriers
• Lot #1	3193	w/ copper	No "exposure"	6 carriers
• Lot #1	2190	w/ copper	Exposed with cleaner	6 carriers
• Lot #1	2190-O	w/ copper	Exposed with cleaner	6 carriers
• Lot #1	2190-I	w/ copper	Exposed with cleaner	6 carriers
• Lot #1	2190-B	w/ copper	Exposed with cleaner	6 carriers
• Lot #1	3193	w/ copper	Exposed with cleaner	6 carriers
• Lot #1	2190	w/ copper	Exposed with quat	6 carriers
• Lot #1	2190-O	w/ copper	Exposed with quat	6 carriers
• Lot #1	2190-I	w/ copper	Exposed with quat	6 carriers
• Lot #1	2190-B	w/ copper	Exposed with quat	6 carriers
• Lot #1	3193	w/ copper	Exposed with quat	6 carriers

Lot #2

• Lot #2	2190	No copper	No "exposure"	4 carriers
• Lot #2	2190-O	No copper	No "exposure"	4 carriers
• Lot #2	2190-I	No copper	No "exposure"	4 carriers
• Lot #2	2190-B	No copper	No "exposure"	4 carriers
• Lot #2	3193	No copper	No "exposure"	4 carriers
• Lot #2	2190	w/ copper	No "exposure"	6 carriers
• Lot #2	2190-O	w/ copper	No "exposure"	6 carriers
• Lot #2	2190-I	w/ copper	No "exposure"	6 carriers
• Lot #2	2190-B	w/ copper	No "exposure"	6 carriers
• Lot #2	3193	w/ copper	No "exposure"	6 carriers

Note 1: The chemical "exposure" and wear will be performed by the Sponsor at the Sponsor facility.

Note 2: Testing should be conducted at the lower certified limit (LCL). Certificate of analysis shall be provided by the Sponsor.

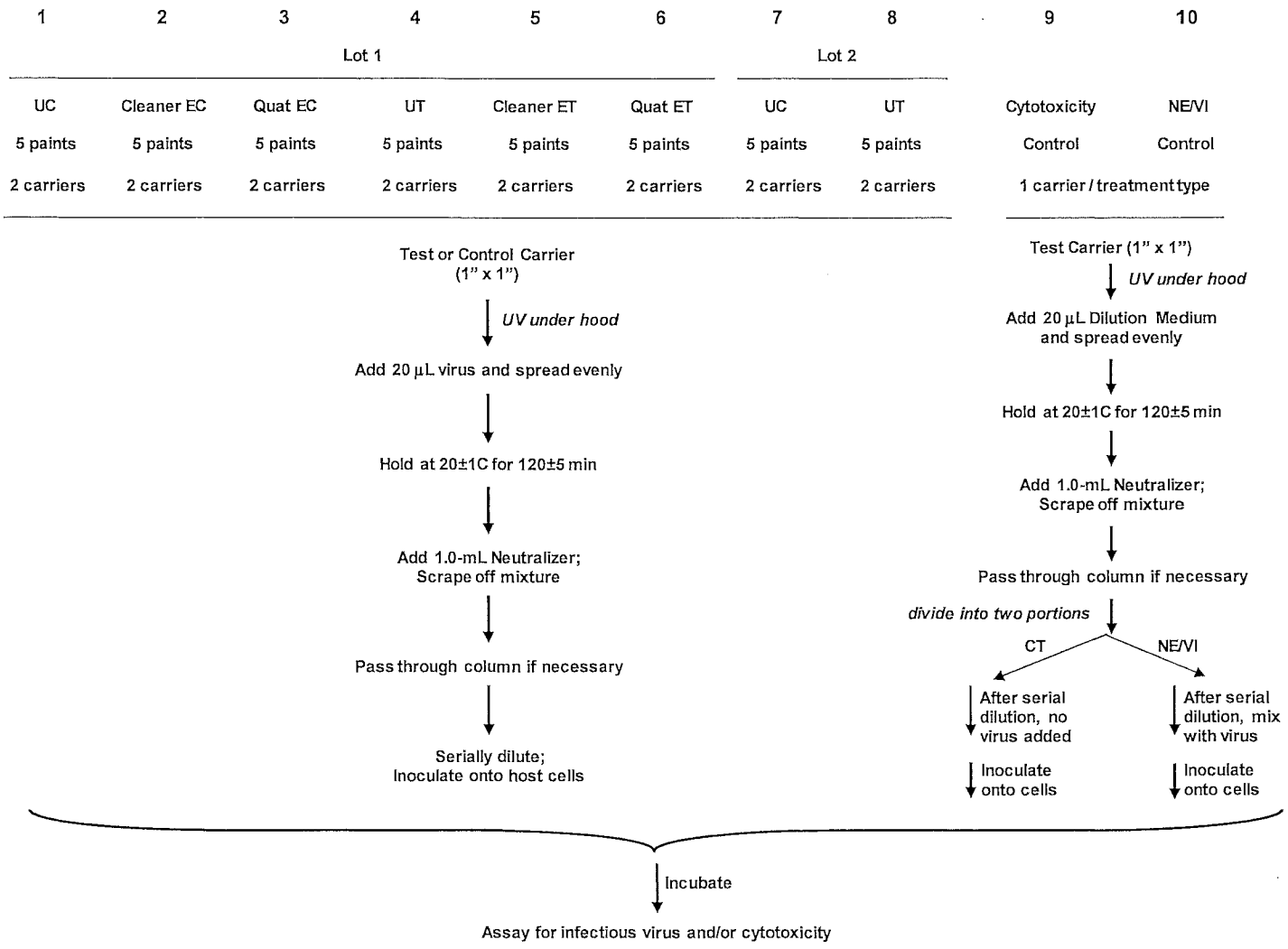
TEST SYSTEM IDENTIFICATION:

All Petri dishes, dilution tube racks, and host-containing apparatus will be appropriately labeled with the following information: virus, host, and test substance and/or project number.

EXPERIMENTAL DESIGN:

All of the procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at Microbac. SOPs and Logs are referred to in the raw data and are required as part of GLP regulations. The study flow diagram is shown in Figure 1, with details described in the following sections.

FIGURE 1



Note: In addition to the above samples, a Glass Plate Recovery Control (GPRC), Viral Stock Titer Control (VST) and Cell Viability Control (CVC) will be performed on each day a coupon sample is analyzed.

A. Inoculum preparation:

Viral stocks are purchased from reputable sources that identify them by scientifically accepted methods and may have been propagated at Microbac. Records are maintained that demonstrate the origin of the virus. The virus stocks are stored at an ultra-low temperature.

Frozen viral stocks will be thawed on the day of the test. Serum will be added to viral stock to achieve an organic load of 5.0% (if not already 5.0%), unless otherwise directed by the Sponsor and pre-agreed by Microbac. If the challenge virus culture is standardized by concentration or dilution, or if a column is used, these manipulations must be documented and reported.

Note: a level of approximately 4.8 – 6.3 Log_{10} virus challenge (as indicated by the plate recovery control load) when there is no cytotoxicity associated with the test substance, or approximately 3.0 – 4.5 Log_{10} beyond the level of cytotoxicity when present, should be achieved whenever possible.

B. Carrier preparation:

The virucidal efficacy testing shall be performed within 5 business days upon receipt of the coupons. All coupons are single use.

Note: Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study.

On the day of testing, the coated test and control coupons will be placed in the biosafety cabinet (hood) for exposure to UV light for 15±2 min on each side to decontaminate the surface prior to testing. After the UV treatment, place each carrier in a sterile Petri dish using forceps with the treated surface facing up.

C. Test:

The test and control carriers should be evaluated on the same day under the same condition. The temperature and relative humidity during the exposure period shall be recorded and reported.

Each coupon (after UV irradiation) will be inoculated with a 20-µL aliquot of viral inoculum using a calibrated pipette. Spread the inoculum across the surface of the carrier to ensure a full coverage of the surface, spreading as evenly as possible and as close to the edge of carrier as possible using a pipette tip.

Place Petri dishes with carriers in a controlled chamber set to 20±1C under ambient relative humidity (RH), with the lid on, for 120±5 min. Note: The exposure time (contact time) of the inoculum to the carrier surface begins immediately upon the deposition of viral suspension (with soil load) onto the carrier.

Following the exposure period, pipette 1 mL of a neutralizer medium onto each carrier. Using a sterile cell scraper, gently scrape the carrier. Pipette the recovery medium back-and-forth of the carrier to ensure the viral particles are homogeneously mixed throughout the medium. Collect the recovery sample and vortex to mix. This post-neutralized sample (PNS) is considered "undilute".

If necessary, the PNS may be passed through a gel-filtration column (e.g., Sephadex column or Sephacryl column) to reduce the cytotoxicity. The columns will be spun for 3 minutes at 1000 rpm. The pass-through samples will be collected and serially tenfold diluted in dilution medium (DM). Selected dilutions will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section.

If no column is used, the PNS will be directly 10-fold serially diluted in DM (for example, 0.5 mL sample + 4.5 mL DM). Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section.

D. Infectivity assay:

The residual infectious virus in all test and control samples will be detected by viral-induced cytopathic effect (CPE).

Selected dilutions of the recovered inoculum/test substance mixture (test samples) and control samples will be added to cultured host cells (at least four wells per dilution, per reaction mixture) and incubated at 36±2°C with 5±3% CO_2 for total 7 – 9 days. The host cells may be washed twice with phosphate buffered saline prior to inoculation. The inoculated culture will be observed and refed with fresh media as necessary, during the incubation period. These activities, if applicable, will be recorded. The host cells will then be examined microscopically for presence of infectious virions. The resulting virus-specific CPE and test substance-specific cytotoxic effects will be scored by examining all test and control samples. These observations will be recorded.

E. Controls:

1. Coated control carriers (unexposed and exposed):

As described above, for Lot #1, unexposed coated control carriers, cleaner-exposed coated control carriers and quat-exposed coated control carriers for each type of base paint will be tested. For Lot #2, unexposed coated control carriers for each type of base paint will be tested.

Note: The mean viral load from the unexposed coated control carriers are used as the input load to compare with the viral load from the treated test carriers to determine the log reduction for each type of test carrier for lot and each type of paint.

2. Neutralizer effectiveness/Viral interference control (NE/VI):

This control will determine if residual active ingredient is present after neutralization and if the neutralized test substance interferes with the virus infection system. This control will be performed for each type of treated test carriers (with or without exposure) for each lot, at one carrier per type (see Table 1 for details).

Each treated test carrier will be processed exactly as the test procedure but in lieu of viral inoculum, DM will be used to inoculate the carrier. Post the exposure time and neutralization, the neutralized DM/test substance mixture will be divided into two portions, one for cytotoxicity control and the other for neutralizer effectiveness/viral interference control and processed as the test.

If columns are used, the sample will be passed through individual columns and the eluate will be serially diluted ten-fold in DM. If columns are not used, the sample will be directly diluted using serial ten-fold dilutions in DM.

The neutralizer effectiveness/viral interference control (NE/VI) sample will be diluted as follows: using dilution test tubes and appropriate pipette, an aliquot of the PNS will be used for making serial 10-fold dilutions in DM (for example, 0.5 mL sample + 4.5 mL DM). Following serial dilution, 0.1 mL of a low titered virus, containing approximately 1,000 – 5,000 infectious units of virus, will be added to 4.5 mL of each dilution and held for a period of no shorter than 30 minutes. Then these samples will be used to inoculate host cells as described for the test procedure.

Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section.

3. Cytotoxicity control (CT):

This control will be performed for each type of treated test carriers (with or without exposure) for each lot, at one carrier per type (see Table 1 for details).

The cytotoxicity sample, acquired from the neutralizer effectiveness/viral interference control run, will be diluted and have no virus added. Selected dilutions will be inoculated and incubated in the same manner as the rest of the test and control samples. These effects shall be distinct from virus-induced cytopathic effects (CPE), which will be evident in the Virus Stock Titer control cultures.

4. Glass Plate recovery control (GPRC):

This control will be performed in a singlet run on each day a coupon sample is analyzed.

Inoculate 20 µL virus onto a 1" x 1 area of a clean glass surface and hold at 20±1C and ambient relative humidity for 120±5 min. Then add 1.0 mL of neutralizer onto the glass surface and recovery virus using a cell scraper. If columns are used for the test carriers, the PNS from this control will also be passed through a column. Serially dilute the sample and selected dilutions will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section. This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

The result from this control will be used to confirm recovery of at least 4.8-log of infectious virus in this control following drying and neutralization.

5. Virus Stock Titer control (VST):

This control will be performed in a singlet run on each day a coupon sample is analyzed.

An aliquot of the viral inoculum used in the study will be directly serially diluted and inoculated onto the host cells to confirm the titer of the stock virus. This control will demonstrate that the titer of the stock virus is appropriate for use and that the viral infectivity assay is performed appropriately.

6. Cell viability control:

This control will be performed in a singlet run on each day a coupon sample is analyzed. It will demonstrate that cells remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the DM employed throughout the assay period. At least four wells of cells will receive only DM and will be incubated and processed with both test and other controls. This will serve as the negative control.

F. Calculation:

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber (Kärber G., Arch. Exp. Pathol. Pharmakol. 1931, 162: 480-483) or other appropriate methods such as Reed and Muench (Am. J. of Hyg. 1938, 27:493). The $TCID_{50}$/carrier, i.e., the viral load per carrier, will be calculated as follows. These analyses will be described in detail in the final report. The test results will be reported as reduction of the virus titer post treatment with the test substance expressed as log_{10}.

The Virus Load ($TCID_{50}$/carrier) will be calculated in the following manner:
Virus Load (Log_{10} $TCID_{50}$) = Virus Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Volume per sample (mL)]

The Log_{10} Reduction Factor (LRF) will be calculated in the following manner:
Log_{10} Reduction Factor = Initial viral load (Log_{10} $TCID_{50}$, per assayed volume and per carrier) – Output viral load (Log_{10} $TCID_{50}$, per assayed volume and per carrier)

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The infectious virus recovered from the GPRC control must be $\geq$ 4.8-log_{10} $TCID_{50}$ units.
- Viral-induced cytopathic effect must be distinguishable from test substance induced cytotoxic effects (if any).
- The Cell Viability Control (assay negative control) must not exhibit virus.

TEST SUBSTANCE EVALUATION CRITERIA:

Production Lot 1 (simulated wear):

- The treated test carriers – exposed and unexposed - must demonstrate an average of ≥ 3 $\log_{10}$ (i.e., 99.9%) reduction - above cytotoxicity if present - as compared to the mean viral load from the unexposed coated control carriers, for each type of base paint

  Note: The mean viral load from the unexposed coated control carriers are used as the input load to calculate log reduction for each type of test carrier.

- The effects of the simulated wear on mean log reduction should be evaluated as the following: the mean log reduction values for the exposed, treated test carriers should be within 0.5 log of the mean log reduction of the unexposed, treated test carriers

- A comparative assessment is also to be made between exposed coated control carriers and unexposed coated control carriers.

Production Lot 2 (no wear):

- The treated test carriers – unexposed - must demonstrate an average of ≥ 3 $\log_{10}$ (i.e., 99.9%) reduction - above cytotoxicity if present - as compared to the mean viral load from the unexposed coated control carriers, for each type of base paint

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, Virginia 20164.

REGULATORY COMPLIANCE AND QUALITY ASSURANCE (GLP studies only):

This study will be performed in compliance with the US Environmental Protection Agency's Good Laboratory Practices (GLP) regulations, 40 CFR 160 (note: information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test substance resides with the sponsor of the study unless otherwise stated).

The Quality Assurance Unit of Microbac will inspect the conduct of the study for GLP compliance. The dates and a description of the phase(s) inspected, and the dates findings are reported to the study management and study director will be included in the final report.

PROTOCOL AMENDMENTS AND DEVIATIONS:

Any protocol amendment(s) and protocol deviation(s) identified will be reported in project sheet(s) and included in the final report.

REPORT FORMAT:

The report will contain all items required by EPA GLP (40 CFR Part 160.185), EPA 810.2000 (2018) and 810.2200 (2018), and be in compliance with EPA PR Notice 2011-3 (replaced PRN 86-5). Microbac employs a standard report format for each test design. Each final report will provide all the information in the citations above including:

- Sponsor identification
- Test substance identification
- Type of assay and project number
- Study start and end time (clock time)
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)
- Certificate of Analysis (for GLP studies only; if provided by the Sponsor)
- List of personnel involved in the study

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report or an electronic copy will be sent to the Sponsor. The original signed final report, or a copy thereof, will be maintained in the study file. If requested, a draft report will be provided to the Sponsor for review prior to finalization of the report.

All raw data, protocol, protocol modifications, test substance records, the final report (or copy thereof), and correspondence between Microbac and the sponsor will be stored in the archives at Microbac Laboratories, Inc., 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test substance; challenge virus and host cell line monolayers used and the type of recovery media employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

REFERENCES

1. U.S. Environmental Protection Agency (EPA), "Interim Method for Evaluating the Efficacy of Antimicrobial Surface Coatings" (10/02/2020),

2. U.S. Environmental Protection Agency (EPA), "Interim Method for the Evaluation of Bactericidal Activity of Hard, Non-porous Copper-Containing Surface Products" (01/23/20)

3. U.S. Environmental Protection Agency (EPA), Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Environmental Surfaces, Guidance for Efficacy Testing, February 2018.

4. U.S. Environmental Protection Agency (EPA), Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Testing Public Health Antimicrobial Pesticides, Guidance for Efficacy Testing, February 2018.

5. U.S. Environmental Protection Agency (EPA), Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, Frequently Asked Questions (FAQ) for OCSPP 810.2000, 810.2100, and 810.2200.

6. ASTM E1053-20, Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces, ASTM International, West Conshohocken, PA, 2011.

7. Corning protocol MRID #51141402 "Protocol for Measuring Virucidal Efficacy of AM Paints".

MISCELLANEOUS INFORMATION:

The following information is to be completed by the sponsor prior to initiation of the study (please check all applicable open boxes):

Please attach the name, lot number, manufacture date and expiration date for the following materials:

Product Lot #1

•	2190	No copper	No "exposure"
•	2190-O	No copper	No "exposure"
•	2190-I	No copper	No "exposure"
•	2190-B	No copper	No "exposure"
•	3193	No copper	No "exposure"
•	2190	No copper	Exposed with cleaner
•	2190-O	No copper	Exposed with cleaner
•	2190-I	No copper	Exposed with cleaner
•	2190-B	No copper	Exposed with cleaner
•	3193	No copper	Exposed with cleaner
•	2190	No copper	Exposed with quat
•	2190-O	No copper	Exposed with quat
•	2190-I	No copper	Exposed with quat
•	2190-B	No copper	Exposed with quat
•	3193	No copper	Exposed with quat
•	2190	w/ copper	No "exposure"
•	2190-O	w/ copper	No "exposure"
•	2190-I	w/ copper	No "exposure"
•	2190-B	w/ copper	No "exposure"
•	3193	w/ copper	No "exposure"
•	2190	w/ copper	Exposed with cleaner
•	2190-O	w/ copper	Exposed with cleaner
•	2190-I	w/ copper	Exposed with cleaner
•	2190-B	w/ copper	Exposed with cleaner
•	3193	w/ copper	Exposed with cleaner
•	2190	w/ copper	Exposed with quat
•	2190-O	w/ copper	Exposed with quat
•	2190-I	w/ copper	Exposed with quat
•	2190-B	w/ copper	Exposed with quat
•	3193	w/ copper	Exposed with quat

Product Lot #2

- 2190 No copper No "exposure"
- 2190-O No copper No "exposure"
- 2190-I No copper No "exposure"
- 2190-B No copper No "exposure"
- 3193 No copper No "exposure"

- 2190 w/ copper No "exposure"
- 2190-O w/ copper No "exposure"
- 2190-I w/ copper No "exposure"
- 2190-B w/ copper No "exposure"
- 3193 w/ copper No "exposure"

Other information:

Active ingredient(s)	Corning Guardian at 0.96 wt% = 3168 ppm CuO
Test material storage	☒ Ambient □ Refrigerated □ Other:_____
Level of active ingredients in testing	☒ Lower Certified Limit (LCL) □ At or below nominal
MSDS provided	☒ Yes □ No
C of A provided	☒ Yes □ No
Contact time	☒ 120 ± 5 min
Contact temperature	☒ Room Temperature (20±1°C)
Organic Load	☒ 5.0% serum in viral inoculum
Study conduct	☒ GLP □ Non-GLP
Report submission	☒ EPA □ Health Canada □ Other: _____
Other instruction	

PROTOCOL APPROVAL BY SPONSOR:

Sponsor Signature: John A Gilbert Date: 11/10/2020

Printed Name: JOHN GILBERT
Chief R+D Officer

PROTOCOL APPROVAL BY STUDY DIRECTOR (Microbac):

Study Director Signature: _____ Date: 12/14/20

Printed Name: Cory Chiasson